A comparative study of the free carbohydrates in healthy and *Phytophthora cinnamomi*-infected avocado root tips

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Root tips of three root-rot tolerant avocado cultivars, Duke 7, G6 and Martin Grande, and a susceptible Edranol cultivar, were inoculated with *Phytophthora cinnamomi* zoospores. Percentages of sugars and starch present in healthy and infected root tips were determined. There were some differences among cultivars in percentages fructose, glucose and sucrose in control roots but no significant difference in percentage total sugars. Infected roots of Duke 7 contained a higher percentage sucrose and G6 a higher percentage fructose and both cultivars contained higher percentages glucose and total sugars than both Martin Grande and Edranol. Infected roots of Martin Grande had lower percentages of fructose, glucose and total sugars, and those of Edranol lower percentages of glucose, sucrose and total sugars than control roots. None of the cultivars showed a significant difference in percentage starch between infected and healthy root tips. This preliminary study shows that further studies are required to determine the role free carbohydrates play in resistance mechanisms.

Keywords: Avocado, *Phytophthora cinnamomi*, resistance, sugars.

Phytophthora cinnamomi Rands is the causal agent of phytophthora root rot, a serious world-wide problem on avocados (Zentmyer 1980). Rootstock selections such as Duke 7, G6 and Martin Grande have been screened and evaluated for their resistance to root rot (Dolan & Coffey 1986; Gabor & Coffey 1990, 1991). Resistance in avocado rootstocks has been described as field resistance (Gabor & Coffey 1990, 1991) however, little is known about the mechanisms of resistance operating in these and other new rootstocks (Gabor & Coffey 1990). Gabor and Coffey (1990) suggested that the resistance of Martin Grande to root rot may be attributable in part to a higher root growth potential and restricted development of *P. cinnamomi* in juvenile roots. Aveling and Rijkenberg (1991) found that Martin Grande attracted significantly less zoospores than the susceptible Edranol and a higher percentage of zoospores failed to germinate on roots of the resistant cultivars, G6, Duke 7 and Martin Grande. Anatomical changes which result in barriers to invasion within root tissues may also be involved in the resistance of several avocado rootstocks (Phillips *et al.* 1987). However, Phillips *et al.* (1987) found that the basis of resistance in calli of avocado cultivars appeared to be largely physiological and (or) biochemical rather than anatomical.

Carbohydrate catabolism in host tissues is accelerated during infection by most parasitic and pathogenic micro-organisms (Asahi *et al.* 1979). One of the principal effects of infection in certain host-pathogen systems is the activation of host systems for repair of injury (Kosuge 1978). Reserve polysaccharides and soluble sugars are mobilised to provide energy for the synthesis of phytoalexins, phenolic compounds, or lignin (Asahi *et al.* 1979). A large but specific set of genes, including defence genes, is positively regulated by sugars (Koch 1996). Horsfall and Dimond (1957) discussed the influence of host sugar content on susceptibility and resistance. They considered, as low sugar diseases, those situations in which low host sugar content confer susceptibility and, as high sugar diseases, those cases where high sugar levels confer susceptibility. *Phytophthora cinnamomi* is a necrotroph (Cahill & Weste 1983) and low sugar diseases are usually associated with necrotrophy (Hughes & Fowler 1953; Horton & Keen 1966). In this paper, a comparative study of free carbohydrates in healthy and *P. cinnamomi*-inoculated avocado root tips, in relation to susceptibility or resistance, was undertaken.

Four-month-old root-rot-resistant avocado cultivars, Duke 7, G6 and Martin Grande and a susceptible Edranol avocado cultivar, were obtained from Westfalia Estates, Duiwelskloof. The seedlings were grown under shade cloth for two years in 30 × 15 cm polythene bags filled with potting soil. Soil moisture was maintained at field capacity by daily watering.

The technique of Marks and Kassaby (1974) was adapted to isolate *P. cinnamomi* from infected soil. A 70 g soil sample was placed in a 250 ml beaker and 150 ml distilled water was added. Avocado leaves were cut into 1 × 1 cm squares, surface sterilised in 1:9 sodium hypochlorite (3.5% m/v):distilled water. The leaf discs were floated on the surface of the water in the beaker for 24 h. The leaf discs were then placed on selective cornmeal agar amended with hymexazol (PARPH) (Tsao & Guy 1977). After incubation for 48 h at 24°C, *P. cinnamomi* isolates were transferred to, and maintained on V-8 juice agar (per litre: 200 ml V-8 juice; 2 g $CaC0_3$; 20 g agar) at 24°C. Sporangia were produced in a mineral salt medium described by Chen and Zentmyer (1970). Zoospore release was achieved by chilling the plates containing the fungal mats for 30 min to 4°C and returning them to 24°C. Most zoospores were released within 0.5–1 h after chilling. Zoospore concentration was calibrated with a haemocytometer and inoculum was used immediately.

Prior to inoculation, the polythene bags containing the avocado seedlings were removed and the roots were washed free of adhering potting soil. The nurse seeds of the Duke 7, G6 and Martin Grande cultivars were excised. Five plants of each cultivar were immersed in a basin containing 25 l of distilled water. Zoospore inoculum was added to the water to give a final concentration of 100 zoospores ml^{-1}. Five control plants of each cultivar were similarly immersed in a basin containing 25 l distilled water but were not inoculated with zoospores. Four hours after inoculation the water in the two basins was replaced and air was bubbled through the water using a Kiho V2 electronic flow control unit to prevent the roots from waterlogging. Young white root tips (*c.* 7 mm-long) were harvested 48 h after inoculation. The inoculation procedure was repeated once. The root tips harvested from each inoculated or control plant were placed in separate petri dishes. The petri dishes and their contents were frozen at -70°C and freeze-dried overnight in a Virtis 10-324 freeze dryer. One hundred and fifty milligrams of freeze-dried root sample per plant was weighed into a centrifuge tube. Five ml 80% ethanol solution was added and the root tips were homogenised using a Virtis '45' homogenizer. The sample was extracted for 30 min in a waterbath at 80°C whilst stirring and centrifuged at 7000 xg for 10 min in a Sorvall RT 6000 refrigerated centrifuge. The supernatant was removed and kept aside and the pellet was re-extracted using 5 ml 80% ethanol for 30 min at 80°C. This sample was again centrifuged at 7000 xg for 10 min. The supernatant fractions were combined and made up to 10 ml with 80% ethanol. The pellets were stored at -20°C for starch analysis. A Seppak C18 cartridge was pre-wetted with 2 ml acetonitrile, flushed with 5 ml distilled water and then 3 volumes of air (15 ml) using a syringe. The supernatant sample was drawn up into a

graduated 10 ml syringe and the syringe was attached to the Seppak cartridge. The first 4 ml of eluent was discarded and the remaining 6 ml was collected. The fraction was then evaporated to 1 ml in a water bath at 32°C using an air blower. Distilled water (0.5 ml) was added to the 1 ml fraction and this was blown with air to dryness in the water bath.

The separation of sugars was done using a Spectra-Physics SP8100 high performance liquid chromatograph with a Waters R401 refractor index detector. The separation column (220 mm × 4.2 mm) was a Millipore carbohydrate column. The solvent used was 80% acetonitrile. The root extracts were passed through a 0.45 µm filter and were afterwards injected onto the column isocratically under the following conditions: flow rate 2.2 ml min^{-1}; injection volume 50 µl. The sugar retention times were: fructose 4.70 min, glucose 5.90 min and sucrose 8.20 min. The measurement was repeated once. A standard curve involving solutions of pure sugar standards was determined for quantitation.

For the starch analysis, the frozen pellet was resuspended in 3 ml distilled water and vortexed. The sample was boiled in a waterbath until dry and then diluted to 10 ml with distilled water. A 0.5 ml sample was pipetted into a centrifuge tube and 0.5 ml acetate buffer (0.02M, pH 4.5) was added to the resuspended tissue. The sample was boiled for 60 min in a waterbath and allowed to cool before adding 1 ml amyloglucosidase (14 units; *Aspergillus niger*: Sigma kit 510-DA). After incubating the sample for 20 h in a waterbath at 45°C, it was boiled for 2 min and centrifuged for 10 min at 7000 xg. A 0.5 ml sample of the supernatant was pipetted into a centrifuge tube and 5 ml combined enzyme-colour reagent solution (Sigma kit 510-DA) was added. The sample was incubated for 30 min at 37°C. The absorbance was read at 450 nm using a Shimadzu UV - 265 recording spectrophotometer and distilled water/acetate buffer/enzyme-colour reagent as the reference blank. The measurement was repeated once.

Rows across Table 1 are a comparative record of the differences in percentage sugars and starch in control (healthy) and inoculated (infected) root tips among cultivars. When control root tips of the various cultivars are compared it was found that Duke 7, Martin Grande and G6 did not differ significantly from Edranol in percentage fructose. However, control roots of Martin Grande and G6 had significantly lower percentage sucrose and glucose, respectively, when compared with the other cultivars. There were no significant differences in percentage total sugars among control root samples of different cultivars, however, Duke 7 had a significantly higher percentage starch. Of the inoculated (infected) root samples, G6 had a significantly higher percentage fructose and Duke 7 and G6 had the highest percentage glucose and total sugars when compared with the other cultivars. Duke 7 had the highest percentage sucrose but did not differ significantly from G6. Martin Grande had the lowest percentage total sugars but did not differ significantly from Edranol. Infected root tips of Edranol had the lowest percentage starch but did not differ significantly from G6. Duke 7 had the highest percentage starch.

Columns within Table 1 record the differences in percentage sugars and starch between healthy and infected root tips of a particular cultivar. There were no significant differences in percentage fructose, glucose, sucrose and total sugars between infected and control root tips of Duke 7. The same results were found for G6 with the exception of percentage glucose which was significantly lower in infected root tips. Percentage fructose and sucrose were significantly more in healthy than infected root tips of Martin Grande and Edranol, respectively. Healthy root tips of these two cultivars also contained significantly higher percentages of glucose and total sugars than infected root tips. None of the cultivars showed a significant difference in percentage starch between infected and healthy root tips.

Table 1 Percentages sugars and starch in healthy (control) and *Phytophthora cinnamomi*-infected (inoculated) avocado root tips

% Sugars or starch	Duke 7	Martin Grande	G6	Edranol
Fructose				
Control	0.03^{*a**x}	0.05^{av}	0.05^{av}	0.03^{axy}
Inoculated	0.01^{ax}	0.02^{bx}	0.04^{ay}	0.02^{ax}
Glucose				
Control	0.46^{ay}	0.37^{ax}	0.46^{ay}	0.44^{ay}
Inoculated	0.33^{ay}	0.18^{bx}	0.35^{by}	0.20^{bx}
Sucrose				
Control	0.20^{ay}	0.19^{ay}	0.13^{ax}	0.19^{ay}
Inoculated	0.20^{ay}	0.12^{ax}	0.15^{axy}	0.12^{bx}
Total sugars				
Control	0.68^{ax}	0.61^{ax}	0.65^{ax}	0.66^{ax}
Inoculated	0.54^{ay}	0.31^{bx}	0.55^{ay}	0.35^{bx}
Starch				
Control	5.10^{ay}	2.90^{ax}	2.43^{ax}	2.82^{ax}
inoculated	4.32^{az}	3.35^{ay}	2.34^{ax}	1.76^{ax}

*Each two successive values down a column, followed by the same letter do not differ significantly according to Duncan's new multiple range test ($P = 0.05$).

**Across a row, values followed by the same letter do not differ significantly according to Duncan's new multiple range test ($P = 0.05$).

There were some differences among cultivars in percentages fructose, glucose and sucrose in control roots, but there was no significant difference in percentage total sugars. This indicates that similar concentrations of these sugars were present in the roots at the time of the experiment although they may be present in different forms.

Kellam and Coffey (1985) and Zilberstein and Pinkas (1987) considered Duke 7 and G6 moderately resistant to root rot. Edranol, on the otherhand, is considered susceptible to *P. cinnamomi*. Dolan and Coffey (1986), using laboratory screening techniques, found that Martin Grande showed the highest level of resistance to *P. cinnamomi* among the cultivars they tested. In this study the percentage fructose, sucrose and total sugars in infected roots of Duke 7 and G6 did not differ from healthy roots. Furthermore, infected roots of Duke 7 contained a higher percentage sucrose and G6 a higher percentage fructose and both cultivars contained higher percentages glucose and total sugars than both Martin Grande and Edranol. Vanderplank (1984) stated that a high sugar content depressed the enzymatic processes involved in the degradation of cell walls and Horton and Keen (1966) hypothesised that in onion pink root, caused by *Pyrenochaeta terrestris*, high host sugar content affected disease development through its repressive effects on pathogen synthesis of cellulase and polygalacturonase. In 1971, Biehn and Dimond (1971) found that monosaccharides depressed polygalacturonase synthesis by pathogens. Borrod (1974), in studies of the parasitism of chestnut by *P. cinnamomi* and the enzyme relations of the fungus, found that the pathogen synthesised a cellulase, but the exact role of

this enzyme in disease development was not determined.

When comparing the differences in percentage fructose, glucose and sucrose between healthy and infected root tips, the infected roots of Martin Grande had lower percentages of fructose glucose and total sugars, and those of Edranol lower percentages of glucose, sucrose and total sugars. Soluble sugars are depleted by wasteful host respiration and by host defence reactions (Kosuge 1978; Asahi *et al.* 1979). Asahi *et al.* (1979) suggested that, in resistance reactions, reduction of soluble sugar levels was probably enhanced and then depressed during infection by a pathogen. They further suggested that in susceptible reactions, reduction of soluble sugar levels may continue because the susceptible host lacks the ability to complete defence reactions quickly. Soluble sugars in infected Martin Grande and Edranol roots may be used to fuel active defence reactions, such as the formation of polyphenols, phytoalexins and other abnormal metabolites.

Further studies of free carbohydrate contents in avocado roots at various time intervals after inoculation with *P. cinnamomi* zoospores and the formation of polyphenols, phytoalexins and other abnormal metabolites are necessary to shed more light on the results reported here. It may be that, in roots of Martin Grande, host metabolism returns from an accelerated to a normal rate after infected cells have completed their defence reactions whilst in roots of Duke 7 and G6, the maintenance of high sugar concentrations in the presence of the pathogen, may be a different resistance mechanism. In roots of Edranol, however, metabolic disturbances may remain uncorrected and the disorderly metabolism of host cells may facilitate a continuing offensive by the pathogen.

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A first record of *Agave decipiens* naturalised in southern Africa

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Agave decipiens Baker is native to the southern parts of Florida, U.S.A. This alien species is reported from South Africa for the first time. In this report the South African plants are described and illustrated, a tube/sepal ideogram is provided and the status of naturalisation is given.

Keywords: adventive alien, Agavaceae, first record, ideogram.

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Agave L., the largest genus in the Agavaceae, comprises about 275 species of rosulate, succulent-leaved perennials. The genus is native to the southern parts of the United States of America, Mexico, Central America and the northern parts of South America (Gentry 1982; Mabberley 1997). Popularly known as century plants or American aloes, a number of species have since the early 1860s been introduced into and eventually became established in amenity horticulture in South Africa. Two taxa, *A. americana* L. var. *americana* and *A. sisalana* Perrine are widely distributed in this country (Figures 1 and 2 in Smith and Mössmer 1996). The distribution of these agaves is largely a reflection of where they have been planted. Although they have shown limited spread from cultivation they reproduce freely, mainly by suckering. They are currently regarded as naturalised and are included in catalogues of problem plants in southern Africa (Wells *et al.* 1986; Henderson 1995).

It was recently noticed that a further species of *Agave* has